By the present amendments, previous Claims 15 through 63 are cancelled in favor of new Claims 64 through 89. New Claims 64 and 65 correspond to previous Claims 17 and 46, which the Examiner indicated were allowed. The remaining claims are dependent on new Claims 66 or 67, respectively.

New Claim 66 is directed to a method for continuously detecting a nucleic acid sequence as amplification reaction proceeds by continuous detection of the change in fluorometric intensity.

New Claim 67 is directed to a method for continuously detecting a specific DNA sequence labeled with a fluorescent donor and acceptor pair, based on a change in fluorescent intensity as cleavage or ligation reaction occurs.

The new dependent claims closely parallel the previous dependent claims. Therefore, these amendments should not introduce any new matter and are introduced in an effort to expedite prosecution.

Turning now to the Office Action, Claims 15, 16, 18 to 20, 32, 35, 56, 57 and 62 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Takahashi et al. It is anticipated that this rejection will not be applicable to any of the current pending claims.

As discussed above, new Claims 64 and 65 correspond to previous Claims 17 and 46, hence are free of the prior art for the reasons of record.

New Claim 66, and Claims 69 through 76, 84 and 85 dependent thereon, are also not anticipated or suggested by Takahashi et al.

This reference described a method wherein a reaction is detected by fluorometric means using a Dnase that results in liberation of a fluorometrically labeled mononucleotides as a cleavage reaction proceeds. Specifically, the reaction involves exonuclease cleavage of a single stranded DNA sequence comprised of the DNA analog poly (dEA). In the reaction of Takahashi, a single stranded DNA is entirely degraded to mononucleotides which are comprised of the DNA analog poly (dEA) that provides "a convenient signal for studying nuclease ... [activity]."

The reference, however, completely fails to teach or suggest a method as recited in Claim 66 or dependent claims wherein a specific DNA sequence is detected continuously as an amplification reaction proceeds based on a change in fluorescence intensity.

The remaining claims all depend from Claim 67 and are directed to a method for continuously fluorometrically detecting a specific nucleic acid sequence, wherein said nucleic acid sequence is labeled with a fluorescent acceptor and donor pair. This also is not taught or suggested by Takahashi et al.

Therefore, all the claims should be free of the §102(b) rejection based on Takahashi et al.

Claims 15, 16, 19-21, 23, 25-30, 32, 37, 43, 45, 47, 51, 52, 55-57 and 59-63 were rejected under 35 U.S.C. §102(e) as being anticipated by Livak et al (USP 5,538,848). This rejection should not be maintained because this reference does not qualify as prior art to the claimed invention. This has been established by the §131 Declaration submitted in the parent application (a copy of which is attached to this Reply), wherein the inventors averred that they had conceived and reduced the invention to practice prior to August 11, 1994 (date of a prior art reference then applied). The reduction to practice was demonstrated by exhibits (notebook pages) also attached hereto. As this information also constitutes evidence of the conception and reduction to practice of the claims therein, this §131 Declaration and exhibits establish that Livak et al is not prior art under 35 U.S.C. §102(e) because its effective filing date of November 16, 1994 is after August 11, 1999. Withdrawal of this rejection is respectfully requested.

Claims 15, 16, 19-35, 37, 39, 41, 43, 45, 47, 49, 51, 52, 55-57 and 59-63 were also rejected under 35 U.S.C. §103 as being obvious over Ashiwara et al (Kokoku 5-15439, March 1, 1993) in view of Livak et al.

Livak et al has been discussed above. Based on the §131 Declaration submitted herewith (and in the parent application) this reference does not qualify as prior art to the claimed invention.

Nor does Ashiwara teach or suggest the claimed invention. As recognized by the Examiner, Ashiwara does not teach continuous fluorometric detection of a specific

nucleic acid sequence during a cleavage or ligation reaction. By contrast, as asserted by the Examiner, "Ashiwara does not teach performance of the method in a continuous fashion."

However a requirement of all the claims is continuous fluorometric detection of a nucleic acid sequence during a cleavage or ligation reaction. Moreover, Ashiwara provides no suggestion or evidence that would allow one of ordinary skill to apply his method in a continuous fashion while a cleavage or ligation reaction proceeds. This is clear, e.g., from page 5, lines 27-29, of the document, and the Examples.

Also, Livak cannot cure the deficiencies of Ashiwara et al as the reference is not prior art to the invention.

Claims 15, 16, 18-33, 35-38, 40, 42, 43, 45, 47, 48, and 51-63 were rejected under 35 U.S.C. §103(a) as being unpatentable over Takahashi et al in view of Livak and further in view of Freifelder et al, and Chow et al.

Takahashi et al has been discussed above. For the reasons noted therein, the reference fails to teach or suggest the continuous fluorometric detection methods of the invention that (i) are effected during amplification of said specific DNA sequence, or (ii) are effected using a specific DNA sequence labeled with a fluorescent donor/acceptor pair.

Livak is not prior art, accordingly it is not specifically addressed.

Freifelder is cited merely to establish that Dnase I and pancreatic Rnase are endonucleases. This reference is otherwise irrelevant to the claims and, therefore, does not compensate for the noted deficiencies of Takahashi.

Chow is cited merely to establish the use of HIV retroviral integrase cleavage assays. It also is otherwise irrelevant to the claimed invention.

Therefore, the claims are patentable over the combination of Takahashi, Livak (not prior art), Freifelder and Chow, which separately or in combination fail to teach or suggest the invention as set forth in Claim 66, and claims dependent thereon (does not suggest continuous fluorometric detection of a DNA produced while an amplification reaction proceeds), or the invention as set forth in Claim 67, and claims dependent thereon (does not suggest continuous detection of a specific nucleic acid sequence attached to an acceptor/donor pair based on change in fluorescence intensity).

Therefore, this rejection should not be maintained against the current claims.

Claims 15, 16, 18-33, 35-38, 40, 42-45, 47-48, and 51-63 were rejected under 35 U.S.C. §103(a) as being unpatentable over Takahashi et al in view of Livak, Freifelder, Chow and Walder et al (WO 89/09284).

Takahashi et al has been discussed above. For the reasons set forth *supra*, the reference does not teach or suggest any of the methods currently claimed.

Livak et not prior art and is not addressed.

Freifelder and Chow are discussed above also. It is acknowledged that they respectively allege that Dnase I and pancreatic Rnase enzymes are endonucleases and that HIV retroviral integrase is useful in nuclease cleavage assays. However, neither reference suggests continuous fluorometric detection of a specific nucleic acid sequence as said sequence is being amplified in an amplification reaction, or the detection of a specific nucleic acid sequence by detecting change in fluorescent intensity of a nucleic acid sequence labeled by a donor/acceptor pair.

Finally, Walder is cited based on its disclosure relating to the technique of catalytic hybridization amplification. However, this reference also does not cure the deficiencies of the already-discussed references as there would be no incentive to combine this disclosure with Takahashi et al (or other references) as there would be no suggestion that the detection reaction of Takahashi et al would be useful for detection of a specific DNA produced during an amplification reaction. Hence, there would have been no reason to combine Walder with Takahashi or the other secondary references to arrive at the claimed invention.

Accordingly, withdrawal of the rejection based on Takahashi in view of Livak and further in view of Freifelder and further in view of How and Walder et al is respectfully requested.

It is anticipated that this Reply should place this case in condition for allowance. A Notice to that effect is respectfully solicited. However, if the Examiner has any

questions concerning this Reply, or any other matters, he is respectfully requested to contact the undersigned so that prosecution may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____

Robin L. Teskin
Registration No. 35,030

P.O. Box 1404
Alexandria, VA 22313-1404
(703) 836-6620

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